Chapter

Strategies to Improve Drug Delivery in Topical PDT

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Abstract

Topical photodynamic therapy (PDT) has been applied to treat premalignant and malignant lesions such as actinic keratosis and non-melanoma skin cancer. A limiting factor of the technique is cream permeation and studies using chemical and physical approaches to overcome it have increased over the years. This chapter is going to explore the main techniques described in the literature used to improve the cream permeation or the photosensitizer (PS) distribution concerning homogeneity. Outcomes-based on animal studies and clinical trials comparing different delivery techniques are going to be presented, highlighting the aspects of invasiveness, costs, harmfulness, and effectiveness of those methods.

Keywords: topical photodynamic therapy, drug delivery, skin cancer, protoporphyrin IX, precursors

1. Introduction

Yet topical photodynamic therapy (PDT) principle is simple and the technique is full of potential to provide an effective treatment for very common malignant and pre-malignant diseases, achieving satisfactory outcomes for a varied number of lesion types with a single approach is not feasible.

Light delivery is successful depending essentially on the ability to provide the best combination of the most suitable wavelength for the drug of choice and the optical properties of the tissue, which determine if light reaches the extension of tissue necessary and if undesired side effects will show up.

Conversely, characteristics on the photosensitizer (PS) accumulation in target tissues are less dependent on direct control of the therapist than light delivery. Further, PS accumulation also defines the light dose (fluency). Therefore, PS accumulation must be properly dealt with in order to provide enhancement of the efficacy of PDT, and presenting the most relevant aspects reported in the literature concerning the strategies to do so is the scope of this chapter.

Topical PDT has been widely applied as a treatment option for pre-malignant lesions such as actinic keratosis (AK) and also for non-melanoma skin cancer (NMSC) lesions [1] such as basal cell carcinoma (BCC) and Bowen disease (BD). The treatment involves the topical application of a PS precursor, followed by the waiting for the drug light interval (DLI) to promote the PS accumulation into the lesion, after which the localized irradiation takes place. Depending on the protocol, this sequence can be repeated with intervals of one week [2–5] or on the same day [6].

The molecules more commonly used worldwide are the aminolevulinic acid (ALA) and its derivative, methyl aminolevulinate (MAL). Both of them can promote protoporphyrin IX (PpIX) endogenous accumulation in cells. The DLI for these drugs can vary from 1 to 4 hours [7], depending on the protocol treatment chosen for the lesion type.

Being clinical PDT still a very empirical field, and since the protocols choices are decisive in determining the therapy outcome, several strategies, both physical and chemical, have been developed aiming to increase the availability of PS *in loco* to increase topical PDT outcome.

2. Drug delivery strategies

Considering the light attenuation by tissue due to the presence of chromophores and to scattering, the wavelength range preferable for PDT is about 650–850 nm, which is known as the 'optical window' for PDT [8]. Once the light penetration can be handled to reach the whole lesion, the remaining concern is to optimize the drug permeation to improve treatment rate success, especially obtaining the improvement of the response of thicker lesions, which shall increase the possibility to make PDT the treatment of choice for those lesions.

Human skin is a complex organ structured to protect the body. The skin layers organization and constitution, as represented in **Figure 1** (*stratum corneum* (SC), epidermis, dermis, and hypodermis) confer its properties of tensile strength and mechanical resistance [9]. For this reason, skin becomes a barrier for the permeation of precursors, which implies an important limitation of topical PDT, with a direct influence on the incubation time and treatment success. The SC is the skin's outermost layer, and it is considered the main barrier to percutaneous absorption.

Although PpIX precursors have a low molecular weight, the PpIX accumulation after as much as three hours of incubation using exclusively topical application of the cream achieves depths up to 1–2 mm. However, the tissue heterogeneities imply in a non-uniform distribution of the produced PpIX, and portions of the lesion do not produce sufficient PS, impairing the treatment effectiveness [10, 11].

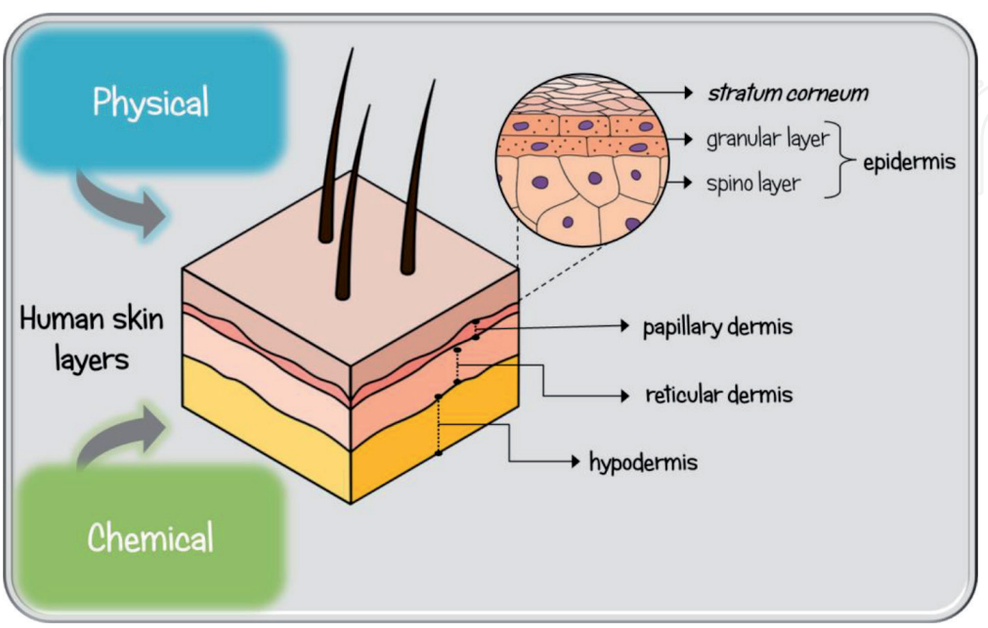

Figure 1.
Representation of human skin layers. Both physical and chemical methods can be explored to overcome the skin barriers and improve topical drug delivery.

Several techniques have been developed to overcome skin barriers and provide penetration enhancement. There are physical and chemical pretreatment strategies to improve drug delivery that can facilitate precursor permeation through skin and into the lesions, allowing PpIX to be produced in deeper layers and also to be distributed more homogeneously.

3. Physical methods

This section presents physical methods based either on creating holes into the skin or on removing the SC to improve drug delivery in topical PDT.

3.1 Tape stripping

This technique consists in applying a tape onto the skin lesions and then to pull it off. By repeating this procedure a number times, which can be repeated using the same piece of tape, part of the SC is removed, reducing it uniformly by about 30%. This procedure is safe, since it is minimally invasive, and involves a low cost to be implemented; as a further advantage, it does not interfere with the intercellular lipids [12–14].

Tape stripping is one of the methods indicated to be applied before PDT; it can be used on patient's lips and also for the preparation of large areas. The recommendation is to choose tapes with strong adhesive properties. According to Christensen *et al*., each area can be stripped 2–4 times [15]. Some studies reported that removing SC by tape stripping significantly increases the PpIX fluorescence in the tissue but does not change the incubation time required for an effective photosensitizer production [16–18].

3.2 Curettage

The curettage can be performed using a sharp ring curette or a scalpel blade. The purpose is to remove keratotic tissue by surface scraping the lesion. The scrape might be performed in a checkered pattern, performing parallel horizontal followed by perpendicular movements. Due to the possibility of subclinical lesion extensions, it is recommended that the curettage is performed using at least 5-mm margins surrounding the lesion to minimize the possibility of the recurrence. The curettage is also indicated to be applied before PDT [13, 15].

This technique is effective, quite inexpensive, and although it can offer light discomfort during the procedure, it is commonly acceptable both by health professionals and patients [19]. However, it is important to mention that there is a risk of bleeding, and when bleeding takes place, it may displace the cream and lead to a decreased PpIX formation, and thus lower treatment efficacy [20]. Despite this, studies reported that curettage associated with PDT increases the cure rate of treatment compared with no-curettage topical PDT. The combination may be repeated successfully for non-cure or recurrence cases without compromising the cosmetic effect or deteriorating skin structures. The treatment has shown a clearance rate of 91% for superficial BCC (131 lesions treated), 93% for thin nodular BCC (82 lesions), and 86% for thick nodular BCC (86 lesions) [21, 22].

3.3 Debulking

Debulking (or deep curettage) is considered a surgical procedure and it is often used in thicker lesions, reducing as much as possible the total volume. This procedure is usually performed either immediately or a few weeks before PDT [15, 23].

Even though it is considered a more invasive procedure compared with the simple curettage, it can be applied without anesthesia. The cosmetic outcome is considered favorable [23] and allows a significant reduction of the BCC thickness, which makes nodular lesions more responsive to the treatment [24, 25].

Whereas the topical PDT efficiency is about 72% when a previous debulking lesion is not applied, for debulked lesions this efficiency can increase up to 92% [23, 26]. Therefore, the debulking associated with PDT is a relevant option for multiple, pigmented, and nodular lesions, improving clinical response [27].

3.4 Ablative fractional laser

This technique consists of using a laser source to create ablated channels on the lesion surface, vaporizing a small portion of the tissue [28]. Usually, lasers with excitation wavelengths in far-infrared spectrum are used, due to the high absorption by the water in the tissue [29, 30]. The main types used are CO2-laser (10,600 nm), Er:YAG-laser (Erbium yttrium aluminum garnet, 2940 nm), and Er:YSSG-laser (Yttrium scandium gallium garnet, 2790 nm) [13].

The depth of the microchannels created varies according to the laser energy. The energies between 32 mJ and 380 mJ generate microchannels with a depth between 300 μm and 2100 μm [31]. Due to the increased skin penetration, this procedure makes PpIX fluorescence both more intense and homogeneous in deeper skin regions, and this can be controlled by adequately adjusting laser energy density [32, 33].

Studies using CO2 laser associated with PDT for lesions treatments showed an increase of about 20% in the clearance of different protocols comparing the treatment without and with the use of laser, respectively [34–36]. Although it is a reliable and effective method, it is also a more invasive procedure with a high potential for skin damage with scarring, discoloration, and even infection. Besides that, the costs of the devices are considered high compared with the abovementioned techniques [37].

3.5 Microneedles

Needles and especially microneedles (MNs) have been used as a method to enhance drug permeation through SC [38]. MNs have the advantages of being minimally invasive, its application is simple, well accepted by the patients, and it is cost-effective [39]. MNs have been developed with lengths up to 900 μm to avoid penetrating in vascular and nervous regions. Therefore, MNs can penetrate the SC without stimulating pain receptors, which is another advantage [40].

The MNs size cannot be seen by patients, thus reducing possible needle phobia. MNs have strong mechanical properties to enable disruption and penetration through the SC, reaching successfully the deeper skin layers without causing any bleeding due to the small length of needles [13].

There are MNs with different shapes (tetrahedron, pyramidal, conical, beveled tip, or tapered cone) that promote different insertion pathways and permeation into the skin. They also can be divided according to the material, such as solid (metallic) or polymeric (hydrogel or dissolving) types, and can be assembled either as a roller or as low-cost patches. The MNs can also be produced with a drug coating on their surface, to facilitate delivery, or hollow, to promote the delivery through channels [13]. The use of MNs rollers for enhancing penetration of topical cosmeceuticals has been well described and transdermal patches can be used easily by anyone [13, 39].

Some studies described a higher formation of PpIX in deeper skin regions when MNs are used and compared with topical application in animal models [41, 42]. A

recent paper by Requena *et al.* explored dissolving MNs containing ALA. The PpIX fluorescence intensity showed to be 5-times higher at 0.5 mm on average compared with cream in *in vivo* tumor mice model [43].

Solid MNs have been commonly used for skin pretreatment before conventional PDT, promoting a good PDT response for actinic keratosis [39]. However, their use for the treatment of BCC or squamous cell carcinoma SCC has not been investigated yet [13].

3.6 Dermograph

The use of dermographs has also been investigated to improve drug delivery. A dermograph is a device currently used in esthetic procedures, which works similarly to a tattoo machine, with simpler handling [44]. The device is composed of a handpiece that couples to different types of needles which oscillate vertically. Usually, both frequency and depth can be adjusted in those devices, according to the application. The holes performed into the skin allow for greater skin penetration of liquids by capillarity [45, 46].

A more homogeneous PpIX distribution and a greater penetration in the tissue were observed for animal model using dermographs compared to the topical application of the cream only [46]. A pilot clinical trial was also performed in nodular BCC lesions using a dermograph for intradermal delivery in PDT, and no recurrence was observed after 28 months of follow-up [46].

3.7 Temperature

Temperature is a parameter with a strong influence on PpIX formation. The increase of the skin temperature (especially in areas that naturally present lower temperatures such as the extremities of the body) during the DLI can improve PDT effectiveness. There are four hypotheses regarding the PpIX formation with the increase of local temperature: increase of ALA penetration in the skin; increase of the conversion rate of ALA to PpIX; increased ALA uptake in the cells due to disruption of the cell membrane, and increase of the amount of skin oxygen due to vasodilation [13, 47].

However, the temperature must be well controlled in order to obtain the desired effect and prevent damage to surrounding tissues [48]. Stringasci *et al.* demonstrated that the most efficient way to increase the production of PpIX and its penetration in the tissue by temperature is to increase the local temperature before applying the cream. This same study reported that the prolonged increase of temperature (during 3 hours) promoted a smaller amount of PpIX accumulation in the skin, unlike other abovementioned hypotheses. That happens because, after increasing the temperature for longer times, cellular metabolism is accelerated which promotes faster elimination of PpIX within the cells [47]. In a clinical study, Willey *et al.* reported the results after PDT treatment of AK on the extremities (head, fingers, and toes) in 18 patients. Skin warming was performed during DLI and was well tolerated, did not promote side effects, and improved PDT efficacy with a clearance rate of 90% after a 1-year follow-up [49].

3.8 Iontophoresis

Iontophoresis is a method based on the use of a voltage gradient applied to an electrolytic formulation, using both a positive (anode) and negative (cathode) electrodes on skin to improve transdermal drug delivery [50]. The advantages of iontophoresis are that it is a non-invasive technique that preserves the skin intact,

providing controlled and targeted delivery of the drug. However, regarding PDT, special attention should be taken to ensure that the photosensitizing drug does not enter the systemic circulation, which can cause unexpected side effects [51].

This technique has a potential to decrease the DLI since *ex vivo* experiments demonstrated PpIX accumulation in human SC were achieved faster compared with topical application only [51]. In addition, studies with patients proved that the use of iontophoresis in clinical protocols is an effective method and reduces the incubation time by 1 h [52].

4. Chemical methods

Currently, only three commercial pro-drugs are licensed for topical PDT use: 5-aminolevulinic acid (ALA, Levulan®), methyl aminolevulinate (MAL, Metvix®), and the nanoemulsion containing ALA (nc-ALA, Ameluz®) [53]. Besides the physical methods to improve the delivery of these drugs, there are chemical approaches that have been explored for drug formulation modifications, and the use of agents that modify the heme cycle or enhance PpIX formation. Thus, the chemical methods to improve PpIX availability mostly treat on the effects over these substances.

4.1 Prodrugs

To enhance ALA penetration through the SC, other prodrugs with longer, more lipophilic carbon chains have been explored [54]. In this context, MAL – which has a methyl group associated with the ALA molecule – already presented more lipophilicity properties and increased selectivity.

The use of even more carbon chains has been tested, such as ethyl-ALA, propyl-ALA, butyl-ALA. The main concern about their use is related to the numerous chemical steps necessary to perform the esterification processes for those molecules [55]. The pro-drug hexyl-ALA has been tested in pre-clinical studies [18, 56]. A pilot clinical trial reported that these molecules presented a greater penetration with lower concentrations compared with ALA or MAL which could reduce adverse reactions and decreases the PDT costs [57]. Despite that, ALA and its ester MAL are still the most commonly chosen and investigated molecules to be used as PpIX pro-drugs for PDT.

One aspect explored in literature for the enhancement of the PpIX prodrugs in cells is the addition of iron chelators. Iron has a crucial role in cells within the heme synthetic route, bound to PpIX molecules to form hemoglobin, thus hindering this process preserves the number of available PpIX molecules within cells. Different chelators have been investigated so far as and, such as desferrioxamine, thiosemicarbazones, pyridoxalisonicotinoyl hydrazone, and di-2-pyridyl-derived iron chelators, among others [58–60]. However, yet thiosemicarbazones have been approved for phase II tests [61], the use of these chelators is still under discussion, due to toxicity versus efficacy issues.

4.2 Differentiation-promoting agents

In addition to the drug choice, different vehicles such as creams, gels, patch systems, lotions, liposomes, nanoparticles, powders, and microemulsions have been developed to overcome the penetration issue [13].

The commercial creams Levulan® and Metvix® are prepared with 20% w/w and 16% w/w concentration of prodrug, respectively. Both of them have been

widely used with acceptable clearance rates and cosmetic outcomes. Aiming to increase the stability of the cream, the molecule of ALA at 7.8% concentration in a nanoemulsion-based gel has been used to produce the Ameluz® formulation. A multicentric study compared this gel formulation with the Metvix® cream in the treatment of AK, demonstrating similar response and tolerance [62].

A patch is an adhesive system used for topical or systemic drug delivery. For PDT, there is a commercial patch (Alacare®) containing 2 mg of ALA per cm^2. A clinical trial using Alacare® for the PDT treatment of actinic cheilitis (AC) demonstrated high clinical efficacy, good tolerability, and favorable cosmetic effects [63]. However, in the case of thick skin lesions without physical pretreatment, this protocol might be less efficient.

4.3 Nanoformulations

Nanotechnology has an important role in the pharmaceutical industry for the development of new formulations. Studies are concentrated on obtaining drugs with more solubility, biodistribution, bioavailability, uptake, and excretion, decreasing drugs' toxicity, [64] and there are different vehicles to promote drug delivery by nanostructures.

Nanoparticles (NP) used to be defined as a particle with a size up to 100 nm in any direction [65]. Lucky *et al.* described the nanoparticles used in PDT based on their performed functions or tasks as PSs, PS carriers, and PS energy transducers [66].

Among the several NP possibilities, liposomes are one of the most used for topical PDT applications. Liposomes are vesicles made up of one or more phospholipid bilayers oriented concentrically around an aqueous compartment to act as drug carriers. This nanostructure has been explored for topical PDT to enhance the PS penetration into the skin while decreasing its absorption into systemic circulation. Examples of PSs currently encapsulated in liposomes for topical PDT are ALA, temoporfin (mTHPC, commercially available as Foscan®), and methylene blue. In most studies, liposomal ALA induced a higher PpIX synthesis than free ALA non-encapsulated delivery [67].

Other structures are more commonly used for systemic applications of PDT, such as nanofibers and nanomicelles [68, 69] but they are off the scope of this chapter, yet their results are also promising for PDT application.

4.4 Thermogenic and vasodilating substances

Thermogenic and vasodilating substances have been reported as promising to favor the permeation of drugs on the skin [70, 71]. In PDT, vasodilation also increases the oxygen supply in the tissue, which can further optimize the effectiveness of the treatment.

A study was carried out investigating the association of the substances: menthol, methyl nicotinate, and ginger associated with ALA and MAL. The association of methyl nicotinate with MAL demonstrated 50% higher PpIX production after three hours of incubation compared with the cream containing only MAL. These preclinical results are promising as a possible strategy for decreasing the DLI and increase the PpIX production in skin lesions [72].

5. Conclusion

This chapter presented several approaches reported in the literature for the improvement of topical PDT outcome. Some of the techniques here presented to

address the task of increasing the availability of PS are increasingly being incorporated to PDT protocols, whereas others are very incipient. Yet, the challenge of making PS available is still an open field, plenty of room for further investigation.

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References

[1] Nguyen K, Khachemoune A. An update on topical photodynamic therapy for clinical dermatologists. J. Dermatolog. Treat. 2019

[2] Cabete J, Rafael M, Cravo M, Moura C, Sachse F, Pecegueiro M. Long-term recurrence of nonmelanoma skin cancer after topical methylaminolevulinate photodynamic therapy in a dermato-oncology department. An. Bras. Dermatol. 2015;90(6):846-50.

[3] Buzzá HH, Moriyama LT, Vollet-Filho JD, Inada NM, da Silva AP, Stringasci MD, et al. Overall Results for a National Program of Photodynamic Therapy for Basal Cell Carcinoma: A Multicenter Clinical Study to Bring New Techniques to Social Health Care. Cancer Control [Internet]. 2019 Jan 26;26(1):107327481985688. Available from: http://journals.sagepub.com/doi/10.1177/1073274819856885

[4] Ramirez DP, Kurachi C, Inada NM, Moriyama LT, Salvio AG, Vollet Filho JD, et al. Experience and BCC subtypes as determinants of MAL-PDT response: Preliminary results of a national Brazilian project. Photodiagnosis Photodyn. Ther. 2014;11(1):22-6.

[5] Peris K, Fargnoli MC, Garbe C, Kaufmann R, Bastholt L, Seguin NB, et al. Diagnosis and treatment of basal cell carcinoma: European consensus–based interdisciplinary guidelines. Eur. J. Cancer. 2019;118:10-34.

[6] Ramirez DP, Moriyama LT, de Oliveira ER, Inada NM, Bagnato VS, Kurachi C, et al. Single visit PDT for basal cell carcinoma – a new therapeutic protocol. Photodiagnosis Photodyn. Ther. [Internet]. 2019 Jun;26:375-82. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1572100019301206

[7] Wan MT, Lin JY. Current evidence and applications of photodynamic therapy in dermatology. Clin. Cosmet. Investig. Dermatol. 2014.

[8] Plaetzer K, Krammer B, Berlanda J, Berr F, Kiesslich T. Photophysics and photochemistry of photodynamic therapy: Fundamental aspects. Lasers Med. Sci. 2009;24(2):259-68.

[9] Khavkin J, Ellis DAF. Aging Skin: Histology, Physiology, and Pathology. Facial Plast. Surg. Clin. North Am. 2011. p. 229-34.

[10] LaRochelle EPM, Marra K, LeBlanc RE, Chapman MS, Maytin E V., Pogue BW. Modeling PpIX effective light fluence at depths into the skin for PDT dose comparison. Photodiagnosis Photodyn. Ther. Elsevier; 2019 Mar;25 (January):425-35.

[11] Peng Q, Soler AM, Warloe T, Nesland JM, Giercksky K-E. Selective distribution of porphyrins in skin thick basal cell carcinoma after topical application of methyl 5-aminolevulinate. J. Photochem. Photobiol. B Biol. 2001 Sep;62(3):140-5.

[12] Pellett MA, Roberts MS, Hadgraft J. Supersaturated solutions evaluated with an in vitro stratum corneum tape stripping technique. Int. J. Pharm. 1997;

[13] Champeau M, Vignoud S, Mortier L, Mordon S. Photodynamic therapy for skin cancer: How to enhance drug penetration? J. Photochem. Photobiol. B Biol. [Internet]. 2019 Aug;197:111544. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1011134418312715

[14] SURBER, Christian; SCHWARB FP., SMITH EW. Tape-stripping technique. Drugs Pharm. Sci. 1999. p. 395-410.

[15] Christensen E, Warloe T, Kroon S, Funk J, Helsing P, Soler AM, et al. Guidelines for practical use of MAL-PDT in non-melanoma skin cancer. J. Eur. Acad. Dermatol. Venereol. 2010;24(5):505-12.

[16] Lesar A, Ferguson J, Moseley H. An investigation of the fluorescence induced by topical application of 5-aminolaevulinic acid and methyl aminolaevulinate at different body sites on normal human skin. Photodiagnosis Photodyn. Ther. 2011 Jun;8(2):97-103.

[17] Goff BA, Bachor R, Kollias N, Hasan T. Effects of photodynamic therapy with topical application of 5-aminolevulinic acid on normal skin of hairless guinea pigs. J. Photochem. Photobiol. B Biol. 1992 Aug;15(3):239-51.

[18] van den Akker JTHM, Iani V, Star WM, Sterenborg HJCM, Moan J. Topical Application of 5-Aminolevulinic Acid Hexyl Ester and 5-Aminolevulinic Acid to Normal Nude Mouse Skin: Differences in Protoporphyrin IX Fluorescence Kinetics and the Role of the Stratum Corneum¶. Photochem. Photobiol. 2000;

[19] See J-A, Shumack S, Murrell DF, Rubel DM, Fernández-Peñas P, Salmon R, et al. Consensus recommendations on the use of daylight photodynamic therapy with methyl aminolevulinate cream for actinic keratoses in Australia. Australas. J. Dermatol. 2016 Aug;57(3):167-74.

[20] Nissen C V., Wiegell SR, Philipsen PA, Wulf HC. Short-term chemical pretreatment cannot replace curettage in photodynamic therapy. Photodermatol. Photoimmunol. Photomed. 2016;

[21] Soler AM, Warloe T, Berner A, Giercksky KE. A follow-up study of recurrence and cosmesis in completely responding superficial and nodular basal cell carcinomas treated with methyl 5-aminolaevulinate-based photodynamic therapy alone and with prior curettage. Br. J. Dermatol. [Internet]. 2008 Jul 18;145(3):467-71. Available from: http://doi.wiley.com/10.1111/j.1365-2133.2001.04407.x

[22] Soler AM, Warloe T, TausjØ J, Berner A. Photodynamic therapy by topical aminolevulinic acid, dimethylsulphoxide and curettage in nodular basal cell carcinoma: A one-year follow-up study. Acta Derm. Venereol. 1999;

[23] Thissen MRTM, Schroeter CA, Neumann HAM. SHORT COMMUNICATIONPhotodynamic therapy with delta-aminolaevulinic acid for nodular basal cell carcinomas using a prior debulking technique. Br. J. Dermatol. 2000 Feb;142(2):338-9.

[24] Christensen E, Mørk C, Foss OA. Pre-Treatment Deep Curettage Can Significantly Reduce Tumour Thickness in Thick Basal Cell Carcinoma While Maintaining a Favourable Cosmetic Outcome When Used in Combination with Topical Photodynamic Therapy. J. Skin Cancer. 2011;

[25] Thissen MRTM, Schroeter CA, Neumann HAM. Photodynamic therapy with delta-aminolaevulinic acid for nodular basal cell carcinomas using a prior debulking technique. Br. J. Dermatol. 2000;142(2):338-9.

[26] Arits AHMM, Mosterd K, Essers BAB, Spoorenberg E, Sommer A, De Rooij MJM, et al. Photodynamic therapy versus topical imiquimod versus topical fluorouracil for treatment of superficial basal-cell carcinoma: a single blind, non-inferiority, randomised controlled trial. Lancet Oncol. 2013 Jun;14(7):647-54.

[27] Souza CS, Neves ABS, Felício LAB, Ferreira J, Kurachi C, Bagnato VS. Optimized photodynamic therapy with

systemic photosensitizer following debulking technique for nonmelanoma skin cancers. Dermatologic Surg. 2007;33(2):194-8.

[28] Prakash S, Kumar S. Fabrication of microchannels: A review. Proc. Inst. Mech. Eng. Part B J. Eng. Manuf. 2015 Aug;229(8):1273-88.

[29] Haedersdal M, Sakamoto FH, Farinelli WA, Doukas AG, Tam J, Anderson RR. Pretreatment with ablative fractional laser changes kinetics and biodistribution of topical 5-aminolevulinic acid (ALA) and methyl aminolevulinate (MAL). Lasers Surg. Med. 2014 Aug;46(6):462-9.

[30] Taudorf EH, Haak CS, Erlendsson AM, Philipsen PA, Anderson RR, Paasch U, et al. Fractional ablative erbium YAG laser: Histological characterization of relationships between laser settings and micropore dimensions. Lasers Surg. Med. 2014 Apr;46(4):281-9.

[31] Haak CS, Farinelli WA, Tam J, Doukas AG, Anderson RR, Haedersdal M. Fractional laser-assisted delivery of methyl aminolevulinate: Impact of laser channel depth and incubation time. Lasers Surg. Med. 2012 Dec;44(10):787-95.

[32] Song HS, Jung S-E, Jang YH, Kang HY, Lee E-S, Kim YC. Fractional carbon dioxide laser-assisted photodynamic therapy for patients with actinic keratosis. Photodermatol. Photoimmunol. Photomed. 2015 Nov;31(6):296-301.

[33] Haak CS, Christiansen K, Erlendsson AM, Taudorf EH, Thaysen-Petersen D, Wulf HC, et al. Ablative fractional laser enhances MAL-induced PpIX accumulation: Impact of laser channel density, incubation time and drug concentration. J. Photochem. Photobiol. B Biol. 2016 Jun;159:42-8.

[34] Alexiades M. Randomized, Controlled Trial of Fractional Carbon Dioxide Laser Resurfacing Followed by Ultrashort Incubation Aminolevulinic Acid Blue Light Photodynamic Therapy for Actinic Keratosis. Dermatologic Surg. 2017 Aug;43(8):1053-64.

[35] Haak CS, Togsverd-Bo K, Thaysen-Petersen D, Wulf HC, Paasch U, Anderson RR, et al. Fractional laser-mediated photodynamic therapy of high-risk basal cell carcinomas - a randomized clinical trial. Br. J. Dermatol. 2015 Jan;172(1):215-22.

[36] Choi SH, Kim KH, Song KH. Er:YAG ablative fractional laser-primed photodynamic therapy with methyl aminolevulinate as an alternative treatment option for patients with thin nodular basal cell carcinoma: 12-month follow-up results of a randomized, prospective, comparative trial. J. Eur. Acad. Dermatology Venereol. 2016 May;30(5):783-8.

[37] Preissig J, Hamilton K, Markus R. Current Laser Resurfacing Technologies: A Review that Delves Beneath the Surface. Semin. Plast. Surg. 2012 Nov;26(03):109-16.

[38] McAllister D V., Wang PM, Davis SP, Park J-H, Canatella PJ, Allen MG, et al. Microfabricated needles for transdermal delivery of macromolecules and nanoparticles: Fabrication methods and transport studies. Proc. Natl. Acad. Sci. 2003 Nov;100(24):13755-60.

[39] Clementoni MT, B-Roscher M, Munavalli GS. Photodynamic photorejuvenation of the face with a combination of microneedling, red light, and broadband pulsed light. Lasers Surg. Med. 2010 Feb;42(2):150-9.

[40] Mikolajewska P, Donnelly RF, Garland MJ, Morrow DIJ, Singh TRR, Iani V, et al. Microneedle pre-treatment of human skin improves

5-aminolevulininc acid (ALA)- and 5-aminolevulinic acid methyl ester (MAL)-induced PpIX production for topical photodynamic therapy without increase in pain or erythema. Pharm. Res. 2010;

[41] Jain AK, Lee CH, Gill HS. 5-Aminolevulinic acid coated microneedles for photodynamic therapy of skin tumors. J. Control. Release. 2016 Oct;239:72-81.

[42] Gracielli Sousa RP, de Menezes PFC, Fujita AKL, Requena MB, Govone AB, Escobar A, et al. Microneedles rollers as a potential device to increase ALA diffusion and PpIX production: evaluations by wide-field fluorescence imaging and fluorescence spectroscopy. In: Choi B, Kollias N, Zeng H, Kang HW, Wong BJF, Ilgner JF, et al., editors. 2014. p. 892614. Available from: http://proceedings.spiedigitallibrary.org/proceeding.aspx?doi=10.1117/12.2040618

[43] Requena MB, Permana AD, Vollet-Filho JD, González-Vázquez P, Garcia MR, Faria CMG, et al. Dissolving microneedles containing aminolevulinic acid improves protoporphyrin <scp>IX</scp> distribution. J. Biophotonics [Internet]. 2020 Sep 27;jbio.202000128. Available from: https://onlinelibrary.wiley.com/doi/10.1002/jbio.202000128

[44] Arbache S, de Godoy CE. Microinfusion of drugs into the skin with tattoo equipment. Surg. Cosmet. Dermatology. 2013;

[45] van der Velden EM, Ijsselmuiden OE, Drost BH, Baruchin a M. Dermatography with bleomycin as a new treatment for verrucae vulgaris. Int. J. Dermatol. 1997;36(2):145-50.

[46] Requena MB, Russignoli PE, Vollet-Filho JD, Salvio AG, Fortunato TC, Pratavieira S, et al. Use of dermograph for improvement of PpIX precursor's delivery in Photodynamic Therapy: experimental and clinical pilot studies. Photodiagnosis Photodyn. Ther. [Internet]. 2019 Nov;101599. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1572100019305344

[47] Stringasci MD, Moriyama LT, Vollet-Filho JD, Bagnato VS. Temperature effect on the PpIX production during the use of topical precursors. Photodiagnosis Photodyn. Ther. 2020 Jun;30:101786.

[48] Yang J, Chen AC-H, Wu Q, Jiang S, Liu X, Xiong L, et al. The influence of temperature on 5-aminolevulinic acid-based photodynamic reaction in keratinocytes in vitro. Photodermatol. Photoimmunol. Photomed. 2010 Mar;26(2):83-8.

[49] Willey A, Anderson RR, Sakamoto FH. Temperature-Modulated Photodynamic Therapy for the Treatment of Actinic Keratosis on the Extremities. Dermatologic Surg. 2014 Oct;40(10):1094-102.

[50] Lemos CN, De Souza JG, Simão PS, Lopez RFV. Iontophoresis improved growth reduction of invasive squamous cell carcinoma in topical photodynamic therapy. PLoS One. 2016;

[51] Gratieri T, Kalia YN. Topical Iontophoresis for Targeted Local Drug Delivery to the Eye and Skin. 2014. p. 263-84.

[52] Choi S-H, Kim T-H, Song K-H. Efficacy of iontophoresis-assisted ablative fractional laser photodynamic therapy with short incubation time for the treatment of actinic keratosis: 12-month follow-up results of a prospective, randomised, comparative trial. Photodiagnosis Photodyn. Ther. 2017 Jun;18:105-10.

[53] Wong TH, Morton CA, Collier N, Haylett A, Ibbotson S, McKenna KE, et al. British Association of Dermatologists

and British Photodermatology Group guidelines for topical photodynamic therapy 2018. Br. J. Dermatol. [Internet]. 2019 Apr 2;180(4):730-9. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/bjd.17309

[54] Kiesslich T, Helander L, Illig R, Oberdanner C, Wagner A, Lettner H, et al. Real-time analysis of endogenous protoporphyrin IX fluorescence from δ-aminolevulinic acid and its derivatives reveals distinct time- and dose-dependent characteristics in vitro . J. Biomed. Opt. 2014;

[55] Zhu W, Gao Y-H, Song C-H, Lu Z-B, Namulinda T, Han Y-P, et al. Synthesis and evaluation of new 5-aminolevulinic acid derivatives as prodrugs of protoporphyrin for photodynamic therapy. Photochem. Photobiol. Sci. [Internet]. 2017;16(11):1623-30. Available from: http://xlink.rsc.org/?DOI=C7PP00203C

[56] Morrow DIJ, McCarron PA, Woolfson AD, Juzenas P, Juzeniene A, Iani V, et al. Hexyl aminolaevulinate is a more effective topical photosensitiser precursor than methyl aminolaevulinate and 5-aminolaevulinic acids when applied in equimolar doses. J. Pharm. Sci. 2010;99(8):3486-98.

[57] Neittaanmäki-Perttu N, Grönroos M, Karppinen TT, Tani TT, Snellman E. Hexyl-5-aminolaevulinate 0·2% vs. methyl-5-aminolaevulinate 16% daylight photodynamic therapy for treatment of actinic keratoses: results of a randomized double-blinded pilot trial. Br. J. Dermatol. [Internet]. 2016 Feb;174(2):427-9. Available from: http://doi.wiley.com/10.1111/bjd.13924

[58] Lui GYL, Obeidy P, Ford SJ, Tselepis C, Sharp DM, Jansson PJ, et al. The iron chelator, deferasirox, as a novel strategy for cancer treatment: Oral activity against human lung tumor xenografts and molecular mechanism of actions. Mol. Pharmacol. 2013;

[59] Yuan J, Lovejoy DB, Richardson DR. Novel di-2-pyridyl-derived iron chelators with marked and selective antitumor activity: In vitro and in vivo assessment. Blood. 2004;

[60] Richardson DR, Milnes K. The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective antiproliferative agents II: The mechanism of action of ligands derived from salicylaldehyde benzoyl hydrazone and 2-hydroxy-1-naphthylaldehyde benzoyl hydrazone. Blood. 1997;

[61] Mrozek-Wilczkiewicz A, Serda M, Musiol R, Malecki G, Szurko A, Muchowicz A, et al. Iron Chelators in Photodynamic Therapy Revisited: Synergistic Effect by Novel Highly Active Thiosemicarbazones. ACS Med. Chem. Lett. [Internet]. 2014 Apr 10;5(4):336-9. Available from: https://pubs.acs.org/doi/10.1021/ml400422a

[62] Dirschka T, Ekanayake-Bohlig S, DominicusR,AschoffR,Herrera-CeballosE, Botella-Estrada R, et al. A randomized, intraindividual, non-inferiority, Phase III study comparing daylight photodynamic therapy with BF-200 ALA gel and MAL cream for the treatment of actinic keratosis. J. Eur. Acad. Dermatology Venereol. [Internet]. 2019 Feb;33(2):288-97. Available from: http://doi.wiley.com/10.1111/jdv.15185

[63] Radakovic S, Dangl M, Tanew A. 5-Aminolevulinic acid patch (Alacare) photodynamic therapy for actinic cheilitis: data from a prospective 12-month follow-up study on 21 patients. J. Eur. Acad. Dermatology Venereol. [Internet]. 2020 Sep 10;34(9):2011-5. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/jdv.16247

[64] Aditya NP, Vathsala PG, Vieira V, Murthy RSR, Souto EB. Advances in nanomedicines for malaria treatment. Adv. Colloid Interface Sci. [Internet].

2013 Dec;201-202:1-17. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0001868613001292

[65] Strambeanu N, Demetrovici L, Dragos D, Lungu M. Nanoparticles: Definition, Classification and General Physical Properties. Nanoparticles' Promises and Risks [Internet]. Cham: Springer International Publishing; 2015. p. 3-8. Available from: http://link.springer.com/10.1007/978-3-319-11728-7_1

[66] Lucky SS, Soo KC, Zhang Y. Nanoparticles in Photodynamic Therapy. Chem. Rev. [Internet]. 2015 Feb 25;115(4):1990-2042. Available from: https://pubs.acs.org/doi/10.1021/cr5004198

[67] Dragicevic-Curic N, Fahr A. Liposomes in topical photodynamic therapy. Expert Opin. Drug Deliv. [Internet]. 2012 Aug 25;9(8):1015-32. Available from: http://www.tandfonline.com/doi/full/10.1517/17425247.2012.697894

[68] Wu H, Chen N, Wu Z-M, Chen Z-L, Yan Y-J. Preparation of photosensitizer-loaded PLLA nanofibers and its anti-tumor effect for photodynamic therapy in vitro. J. Biomater. Appl. [Internet]. 2013 Feb 15;27(6):773-9. Available from: http://journals.sagepub.com/doi/10.1177/0885328211425706

[69] Jang W-D, Nakagishi Y, Nishiyama N, Kawauchi S, Morimoto Y, Kikuchi M, et al. Polyion complex micelles for photodynamic therapy: Incorporation of dendritic photosensitizer excitable at long wavelength relevant to improved tissue-penetrating property. J. Control. Release [Internet]. 2006 Jun;113(1):73-9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0168365906001131

[70] Boelsma E, Anderson C, Karlsson AMJ, Ponec M. Microdialysis technique as a method to study the percutaneous penetration of methyl nicotinate through excised human skin, reconstructed epidermis, and human skin in vivo. Pharm. Res. 2000;17(2):141-7.

[71] Sivamani RK, Stoeber B, Wu GC, Zhai H, Liepmann D, Maibach H. Clinical microneedle injection of methyl nicotinate: stratum corneum penetration. Ski. Res. Technol. 2005 May;11(2):152-6.

[72] Stringasci MD, Vollet-Filho JD, Moriyama LT, Kurachi C, Bagnato VS. Investigation of protoporphyrin IX production induced by aminolevulinic acid combined with thermogenic and/or vasodilator substances. In: Hasan T, editor. 17th Int. Photodyn. Assoc. World Congr. [Internet]. SPIE; 2019. p. 216. Available from: https://www.spiedigitallibrary.org/conference-proceedings-of-spie/11070/2525696/Investigation-of-protoporphyrin-IX-production-induced-by-aminolevulinic-acid-combined/10.1117/12.2525696.full